Effectiveness of Glues Used for Harmonic Radar Tag Attachment and Impact on Survival and Behavior of Three Insect Pests

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ABSTRACT The ability of three cyanoacrylate glues to ensure a durable bond between the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), the plum curculio, *Conotrachelus nenuphar* (Herbst), or the corn rootworms (Western Corn Rootworm, *Diabrotica virgifera virgifera* LeConte and Northern Corn Rootworm, *Diabrotica longicornis* Smith and Lawrence) and the harmonic radar tag without impact on behavior and survival was assessed as part of a study on the use of harmonic radar technology to track these insect pests. Droplets of 0.1 mg of Krazy Glue, Loctite, and Bowman FSA applied to the pronotum had no effect on the survival of the Colorado potato beetle or plum curculio after 5 and 7 d, but caused >40% mortality after only 4 h with both the western and northern corn rootworms. The three glues created an effective bond lasting 4–5 d between the harmonic radar tag and the Colorado potato beetle in >85% of cases and the plum curculio in almost 50% of cases. There was no detectable impact of the glue treatment on feeding or walking behavior of the Colorado potato beetle. Analysis of the same behaviors with the plum curculio showed no impact on the ability to walk on a vertical surface, the speed of travel, or the duration of travel. There was no significant impact on feeding by female plum curculio but indication that males treated with Krazy Glue fed less. Overall, results quantified the effectiveness of the cyanoacrylate glues at providing a durable bond with no significant impact on mobility or behavior of the Colorado potato beetle or plum curculios. However, the toxicity of the glues against the corn rootworms suggests that similar toxicity or sublethal effects may exist with other insects.

KEY WORDS adhesives, bonding, cyanoacrylate, behavior, ethological software

Harmonic radar is becoming a useful tool to track insect dispersal. Essentially, harmonic radar systems track insects by illuminating a tag on the target insect with a high-power microwave source and recapturing the second harmonic of the incidental signal reradiated from the tag (Boiteau and Colpitts 2004). The movement of the insect can be tracked through this exchange of signals between the radar and the tagged insect. The microwave signal may be reduced or interrupted by vegetation but can be recovered as long as the insect has not moved outside of the range of the microwave source. Different harmonic radar systems to track insects have been developed around the same concept, but they all require the fixation of tags on individual insects (Riley and Smith 2002). The size and weight of the tags must be very small so that they do not alter normal movement of the insects in the air (Roland et al. 1996, Riley et al. 1998) or on the ground (O'Neal et al. 2004).

A variety of procedures, usually involving glues, have been used to attach the tags to the body of the target insects. To the best of our knowledge, nobody has addressed the possible impact of these glues on the normal movement of the tagged insects. Attachment methods have generally been selected by trial and error to meet the immediate requirements of the study. For example, the free movement of bees in and out of their hives required a method that allowed the tags to be mounted on and off the target insect. Discs, commercially available for the marking of queen bees, were attached to the dorsal surface of the thoraxes using the adhesive provided by the manufacturer and served as a platform to which the matching transponder base could be quickly fixed using a preattached disc of double-sided sticky tape (Osborne et al. 1999, Capaldi et al. 2000, Riley and Smith 2002).

The types of adhesive used have varied considerably (Roland et al. 1996, Lövei et al. 1997, Riley et al. 1998, Svensson et al. 2001, Piper and Compton 2002, O'Neal et al. 2004), but cyanoacrylate glues have been recurrent (Mascanzoni and Wallin 1986, Wallin and Ekbom 1994, Boiteau and Colpitts 2004, Brazee et al. 2005, Wikelski et al. 2006). The cyanoacrylate glues

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are generally available, easy to use, and provide fast bonding.

The chemically complex glues needed to obtain a strong bond between the harmonic radar tags and the insects create an increased risk of sublethal effects on the dispersal behavior of the insects.

Essentially, researchers, through trial and error, have developed procedures to attach tracking tags that have minimal effect on the dispersal and general behavior of the insect and that remain bonded to the insect long enough for the study to be carried out. The increasing use of the harmonic radar technology to acquire ecological information (Reynolds et al. 2007) makes it more important than ever to measure any possible impact of tag attachment methods on the dispersal behavior of target insects, i.e., walking in our case. Tags must be durable and have no or minimal effect on the survival and behavior of the target insect. The durability of the bond between the radar tag and the insect depends on the glue and the surface of the insect to which it is attached.

More specifically, the objective of the study was to determine whether cyanoacrylate glues can provide long lasting bonds between harmonic radar tags and three target coleopteran pests (the Colorado potato beetle, the plum curculio, and the corn rootworms) with no or minimal impact on survival, feeding activity, and mobility. These insects were chosen because they can serve as models for the many pests for which an improved knowledge of movement and orientation is vital to the development of effective monitoring systems or management plans. They are all economically important pest species of three different crops/landscapes: potato (Colorado potato beetle), corn (corn rootworm), and orchards (plum curculio). A harmonic radar detection system could provide much needed information regarding Colorado potato beetle (Boiteau et al. 2003, Hoy et al. 2008), corn rootworm (Meloche et al. 2001, Meloche and Hermans 2004), and plum curculio (Leskey and Wright 2004, Vincent et al. 2004, Chen et al. 2006) movement in real time and over a range of distances and locations. The possible impact of tag weight and size on travel through the natural environment will be considered elsewhere.

Materials and Methods

Glue Treatments

All tests were carried out using three commercially available cyanoacrylate-based glues: (1) Instant Krazy Glue, Advanced Formula, with >70% by weight of ethyl-2-cyanoacrylate (Elmer's Product Canada, Scarborough, Ontario, Canada), (2) SuperGlue by Loctite with >60% by weight of ethyl-2-cyanoacrylate, 5–10% by weight of poly (methyl methacrylate), and 0.1–0.5% by weight of hydroquinone (Henkel Canada, Brampton, Ontario, Canada), and (3) Bowman FSA Adhesive (Bowman glue) with >60% by weight of ethyl-2-cyanoacrylate, 10–30% by weight of poly (methyl methacrylate), and 0–1% by weight of hydroquinone (Barnes Group, Cleveland, OH).

Colorado Potato Beetle

A test to establish the effect of the three types of glue and a water control on the survivorship, feeding activity, and mobility of the Colorado potato beetle and the persistence of the electronic tags on the insects was carried out at room temperature (≈23°C) at the Potato Research Centre of Agriculture and Agri-Food Canada, Fredericton, New Brunswick, Canada, over a period of 5 d.

Adult Colorado potato beetles of the same age and 1:1 sex ratio were taken from the breeding colony at the Potato Research Centre and maintained under similar conditions on potato cultivar Shepody. Beetles were sexed according to Rivnay (1928). Dipole tags used in the treatments were made up of AWG 34 copper-coated steel wire measuring 2 and 6 mm on either side of a 1.0-mm-diameter loop (2.4 ± 0.0000547 mg, $N = 5$) and were attached to the pronotum of adult Colorado potato beetles using a droplet of glue. The pronotum was lightly sanded before applying the glue to remove waxes and increase the quality of the bond (Boiteau and Colpitts 2001). There were 5 beetles per treatment and four replicates for a total of 20 insects per treatment. Beetles for each replicate were held in a 2-liter plastic container with a compound potato leaf placed in a flower pick filled with water. Containers were closed with meshed lids to prevent any potential accumulation of toxic fumes from the glue used in some of the treatments. Beetles were examined every day to determine the number of tags that had fallen off, beetle mortality, beetle mobility, and level of defoliation. Mobility was assessed by observing the beetles in each replicate for 2 min for visible signs of intoxication such as difficulty to move forward and instability. Mobility was recorded as the percentage of beetles in each container displaying the ability to walk at a normal pace and in a stable manner. Similar size leaves were placed daily in each container so that defoliation could be estimated visually from the number of total or partial leaflets missing.

The weight of the droplets of glue used in the test was estimated by applying Krazy Glue to aluminum weigh boats in the same manner as to the Colorado potato beetle and measuring weights before and after for 10 droplets. Weights were measured using a precision balance (Sartorius BP110S, Data Weighing Systems, EIK Grove, IL, USA).

A single-factor analysis of variance (ANOVA) was applied to the daily percentage of defoliation and to the mean number of fallen harmonic radar tags on day 5 (SAS Institute 2001).

Plum Curculio

Survivorship, feeding activity, and mobility tests were carried out on the multivoltine population of the plum curculio at the USDA Appalachian Fruit Research Station (Kearneysville, WV). Similar survivorship and tag attachment tests were carried out on the northern univoltine population of the plum curculio at the Agriculture and Agri-Food Canada Horticultural

Research and Development Centre, Saint-Jean-sur-Richelieu, Quebec, Canada.

Adult multivoltine plum curculios were reared according to Amis and Snow (1985) from larvae collected from dropped infested peaches and plum. Immediately after emergence, adults were held in groups of 100 in an environmental chamber for ≈3 wk at 25°C and 14 L:10 D. Adults were sexed according to the methods of Thomson (1932). Adults were returned to the chamber in single sex groups of ≈40 individuals held in wax-coated cups (473 ml) with a clear plastic lid and provided with a source of water (wetted cotton dental wick) and green thinning apples as a food source.

For the tests, a drop of glue was applied to the pronotum of each adult using a 0.1–2.5 Ependorf pipette tip attached to the commercial adhesive dispenser. Water was used as a control and applied using a 0.1–2.5 Ependorf pipette tip attached to 3 ml low-density polyethylene dropper bottle (Wheaton Scientific Products, Millville, NJ). Adults were positioned upright in an ≈30-ml clear plastic cup (Jetware, Hatfield, PA) to allow the glue or water drop to dry. After drying, each adult was held separately, at room temperature, in a wax-coated cup (473 ml) with a clear plastic lid and provided with a source of water (wetted cotton dental wick) and a green thinning apple as a food source. For the survivorship and feeding activity tests, the number of live adults and the number of feeding punctures per fruit was recorded 7 d later. Data were subsequently analyzed using the General Linear Model (GLM) procedure (SAS Institute 2001) to construct ANOVA tables for the total number of feeding punctures per fruit. Dependent variable data were not transformed because homogeneity-of-variance assumptions were not violated according to Levene's test. If the GLM indicated significant differences, multiple comparisons were calculated using Tukey's honestly significant difference (HSD) at $P < 0.05$.

The possible effect of the adhesives on the horizontal walking activity of the treated multivoltine plum curculio was assessed in test arenas consisting of 100 by 15-mm plastic petri dishes (Becton Dickinson Labware, Franklin Lakes, NJ) with a single, circular sheet of filter paper covering each arena base. Dishes were lidded to contain single test subjects, and six arenas were tested simultaneously. To aid in detection of test subjects and limit glare, trials were conducted in a darkened room, and arenas were backlit using a fluorescent Canon video visualizer stand (model RE-350; Canon, Canon USA Inc., Lake Success, NY). Images were captured using a Canon digital video recorder (12× zoom, 5.4–65 mm, 1:1.8) suspended directly above the array of test arenas. Movement tracks were captured live using Noldus EthoVision software (Version 3.1.16; Noldus Information Technologies, Wageningen, The Netherlands), and each trial consisted of a 15-min recording duration at a capture rate of five samples per second. To separate subject insects from the background, gray scaling was used as the detection method, wherein backlit subject insects always appeared darker than the arena base. Noise thresholds were updated manually before each trial; subject insects were placed at the center of an arena and tracked on capture of a detection area from 3 to 100 pixels (the maximum area of a mobile subject was recorded at 85 pixels). If lost during a trial, the movement track was recovered by interpolating the track between the recapture position and the last known position. For this test, the whole surface of each arena was contained in a single acquisition zone.

The mobility of each subject was evaluated 24 and 48 h after application of the glue or water drop to the pronotum. A total of 20 male and 20 female adult plum curculios were evaluated for each treatment. For analysis, three parameters were calculated: total distance moved (cm), total duration of movement (s), and mean velocity (cm/s). To eliminate artificial accumulation of distance moved caused by shifting of the recorded center of the subject insect's mass (cursor fluctuation), an input filter was added based on the subject insect moving a minimum of 0.5 cm (the average length of a univoltine adult plum curculio; C. Vincent, unpublished results). For duration of movement, a subject insect was considered to commence movement on reaching a velocity of 0.5 cm/s and was recorded as no longer moving when velocity dropped below 0.1 cm/s. Accuracy of the input filter for total distance moved and the parameter properties for total duration of movement were validated by correlating manually recorded movement with video capture outputs. Another parameter, maximum velocity (cm/s), was used to separate erroneous track recordings. Data were subsequently analyzed using the GLM procedure (SAS Institute 2001) to construct ANOVA tables for the total distance moved (cm), duration of movement (s), and mean velocity (cm/s). The models included sex and treatment as class variables. Dependent variable data were not transformed because homogeneity-of-variance assumptions were not violated according to Levene's test.

The possible effect of the adhesives on the vertical mobility of adult multivoltine plum curculio was evaluated 3–7 d after application of glue or water drop to the pronotum and after mobility trials at 24 and 48 h. Each adult was placed inside at the base of a 30-cm-tall clear Plexiglas cylinder with an inner diameter of 7 cm. To aid in detection of test subjects and facilitate upward movement by adults, trials were conducted in a darkened room, and cylinders were illuminated from above using a bank of fluorescent lights 25 cm above the top of the cylinders. The duration of each trial was 5 min, and each test subject was evaluated in three separate trials conducted consecutively. If a test subject reached a height of 20 cm, it was gently prodded to induce it to drop to the cylinder floor to allow for further climbing during the test period. Recorded parameters for each test subject included detection of upward movement as well as the total distance moved and the number of times it reached the top of the cylinder. Data were subsequently analyzed using the GLM procedure (SAS Institute 2001) to construct ANOVA tables for the total distance moved (cm). The models included sex and treatment as class variables. Dependent variable data were not transformed because homogeneity-of-variance assumptions were not violated according to Levene's test. To determine whether the likelihood of movement by a particular adult was related to sex or treatment, a logistic regression model was constructed using a stepwise procedure.

To determine the mean weight of the glue droplet applied to the pronotum, adults were kept individually in ≈30-ml clear plastic cups (Jetware) and placed in a −20°C freezer. They were removed ≈24 h later and allowed to desiccate on the laboratory bench under ambient laboratory conditions of ≈22°C and ≈30% RH. After ≈48 h, each adult was weighed individually using Mettler AT20 Fact analytical balance. Next, a drop of glue was applied to the pronotum of each adult using a 0.1–2.5 Ependorf pipette tip attached to the commercial adhesive dispenser. Adhesive treatments (N = 15) included Bowman FSA, Krazy Glue, and Loctite. Untreated adults (N = 15) served as a control. Treated adults were positioned upright in an ≈30-ml clear plastic cup (Jetware) to allow the glue to dry. Treated and untreated adults were weighed a second time. Untreated adults served to provide an additional water loss correction factor based on pre- and post-treatment weights; this value was added to the weights obtained for each individual for all glue treatments.

The effect of Krazy Glue and Bowman FSA glues on the integrity of the bond between the harmonic radar tag and the univoltine plum curculio was determined using 15 and 13 adults, respectively, collected in an unsprayed orchard of the Agriculture and Agri-Food Canada experimental farm in Frelighsburg, Quebec, Canada. Adults were kept in 1-liter jars, at 22°C, 70%.RH, and 16:8 L:D in a growth chamber and were fed with apple pieces changed twice a week. The dipole tags, similar to those of Colpitts and Boiteau (2004), consisted of a 2-mm proximal pole followed by a 1-mm-diameter loop and a 6-mm-pole made of AWG 34 copper wire. A drop of glue was applied with a wooden toothpick on the pronotum of adults individually immobilized between index and thumb. The tags were held in the drop of glue until dry. Adults were put in 118-ml containers (Solocup, Urbana, IL) with a fresh apple piece. The containers were capped with muslin held in place with a pierced lid to allow air exchange. Apple pieces were changed 3 d after tag gluing. Survival of adults was monitored for 12 d, and integrity of antennae on pronotum was monitored for 5 d.

The weight of Krazy Glue droplets deposited on the pronotum of adult univoltine plum curculio was determined from 10 individuals using an analytical balance (model TE214S; Sartorius AG, Goetingen, Germany). Ten copper antennae were weighted individually with the same balance. Antennae were glued on the pronotum of adults. The weight of the glue was determined by subtracting from the total weight of one adult with a glued antenna, minus the weight of the unglued antenna and the beetle weight before tag attachment.

Corn Rootworm

Tests on the Northern and Western corn rootworm were conducted at the Agriculture and Agri-Food Canada Eastern Cereal and Oilseed Research Centre, Ottawa, Ontario, Canada. Adults used in the study were collected in July and October 2007 from corn fields at the Eastern Cereal and Oilseed Research Centre.

The glue weight was determined by applying a droplet of Bowman FSA, with a 00 insect pin, on the pronotum of 10 Northern corn rootworm adults, under a magnifying glass, and letting it dry for at least 2 min before lifting and weighing the hardened plate of glue on a precision balance (Mettler H20).

For the survivorship test, carried out at room temperature, each glue treatment and a water control was applied, as described above, to five groups (replicates) of 10 Western corn rootworm and 10 Northern corn rootworm placed in 20-cm petri dish cages with organza-covered ventilation holes on the top, side, and bottom (modified from http://insected.arizona.edu/gg/resource/clip.html).

Results

Colorado Potato Beetle

Survivorship and Feeding Activity. All Colorado potato beetles tested survived throughout the 5-d period regardless of the glue treatment. The percentage of potato leaf defoliation varied but did not differ significantly between treatments on any of the 5 d (Table 1). The mean weight of the drop of glue used to hold the tag was 0.12 ± 0.013 mg.

Mobility. The mobility of the Colorado potato beetles tested remained unaffected (100% mobility) by the treatments throughout the 5 d of observation.

Radar Tag Attachment. The strength of the bond was high and statistically similar between glues, with 95, 85, and 95% of tags remaining on the potato beetles after 5 d for Krazy Glue, Loctite, and FSA, respectively (F = 1.333; df = 2,9; P = 0.311; Table 1). Most of the tags that separated from the beetles were found lodged into the openings of the screened lids. Failure of the glue as it aged is unlikely. The tags were probably levered off after persistent attempts by the beetles to liberate themselves.

Plum Curculio

Survivorship. There was no significant difference between the weight of glues applied on the multivoltine plum curculio for any of the treatments (F = 0.44; df = 2,42; P = 0.64). The mean weight of Bowman, Krazy Glue, and Loctite droplets applied to the pronotum of adults were 0.160 ± 0.019, 0.137 ± 0.019, and 0.162 ± 0.022 mg, respectively. The mean weight of Krazy Glue droplets applied on the univoltine plum curculio was similar to that of the multivoltine plum curculio at 0.15 ± 0.034 mg.

Survivorship of females and males was 100% for all glues over a 7-d trial for the multivoltine plum curculio

Table 1. Comparison of the potential effect of three commercially available glues on feeding behavior (defoliation %) and effectiveness at holding a 2:6-mm harmonic radar tag on the pronotum of the Colorado potato beetle (mean no. of tags lost per replicate) over a 5-d period

Treatment	Mean defoliation (%) ± SE				
	Day 1	Day 2	Day 3	Day 4	Day 5
Krazy Glue	87.5 ± 7.77	90.0 ± 10.0	78.8 ± 8.51	96.2 ± 2.39	75.0 ± 18.37
Loctite	65.0 ± 10.21	90.0 ± 0.00	77.5 ± 5.95	70.0 ± 11.55	87.5 ± 4.33
Bowman FSA	67.5 ± 12.50	98.3 ± 1.67	87.5 ± 3.23	92.5 ± 3.23	85.0 ± 35.36
Control (sanded)	97.5 ± 2.50	95.0 ± 0.00	65.0 ± 11.7	90.0 ± 7.07	83.8 ± 6.57
Control (not sanded)	60.0 ± 17.08	96.7 ± 1.67	75.0 ± 15.4	87.5 ± 8.29	86.3 ± 2.39
df	4,15	4,10	4,15	4,15	4,15
F	2.106	0.697	0.662	1.928	0.294
P	0.131	0.611	0.628	0.158	0.878
	Mean number of tags missing ± SE				
Krazy Glue	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.05	0.05 ± 0.05a
Loctite	0.10 ± 0.05	0.10 ± 0.06	0.15 ± 0.05	0.15 ± 0.05	0.15 ± 0.05a
Bowman FSA	0.05 ± 0.05	0.05 ± 0.05	0.05 ± 0.05	0.05 ± 0.05	0.05 ± 0.05a

Means followed by the same letter within a column are not statistically different (Tukey test, $P < 0.05$).

and for Bowman FSA and Krazy Glue over a 12-d trial for the univoltine plum curculio.

Feeding Activity. The type of glue had no significant effect on the mean number of feeding punctures per fruit ($F = 0.69$; df = 3,76; $P = 0.56$) for female multivoltine plum curculio, but a difference was detected for males ($F = 3.36$; df = 3,76; $P = 0.02$) with significantly fewer feeding punctures detected on fruit fed on by males treated with Krazy Glue than with water (Table 2). The number of male multivoltine plum curculio feeding was lower than in the control for all glues.

Mobility. In the absence of statistical differences in the horizontal mobility of male and female multivoltine plum curculios exposed to the glues for 24 or 48 h, data were combined (Table 3). The mean total distance moved ($F = 1.84$; df = 4,155; $P = 0.12$), mean velocity ($F = 0.42$; df = 4,155; $P = 0.79$), and duration of movement ($F = 0.81$; df = 4,155; $P = 0.52$) were not significantly affected by the treatments after 24 h. The mean total distance walked ($F = 0.20$; df = 4,155; $P = 0.94$), mean velocity ($F = 2.12$; df = 4,155; $P = 0.08$), and duration of movement ($F = 0.14$; df = 4,155; $P = 0.96$) were not significantly affected by the treatments after 48 h.

In the absence of statistical differences in the vertical mobility of male and female multivoltine plum curculio exposed to the glues, data were combined (Table 4). The model for mean total vertical distance traveled ($F = 1.66$; df = 4,155; $P = 0.16$) was not significant. Similarly, the likelihood of movement by any of the test subjects was not explained by treatment or sex using logistic regression. For all treatments, >30% of individuals moved in vertical arenas, with 2.5–15.0% of the curculios reaching the top (Table 4).

Radar Tag Attachment. The percentage of univoltine plum curculios with tags remaining attached to the pronotum dropped sharply in the first 24 h to 53% ($N = 15$) with Krazy Glue and 46% ($N = 13$) with Bowman FSA and remained the same during the following 72 h before falling to 33 and 38%, respectively, on the fifth day.

Corn Rootworm

Survivorship. The cyanoacrylate glues tested substantially affected the survival of both species of corn rootworm. Seventy percent or more of the Western corn rootworm and 80% or more of the Northern corn rootworm adults died within 4 h of the application of any one of the three glues tested compared with none in the control (Table 5). The high mortality made it impossible to assess the impact of the glue on feeding activity or mobility of either species.

The mean weight of Bowman glue droplets applied to Northern corn rootworm was 0.115 ± 0.014 mg.

Table 2. Feeding (%; $N = 20$) and mean ± SE no. of punctures per fruit for female and male multivoltine plum curculios after application of three commercially available glues

Treatment	Female		Male	
	Percent feeding	Mean number per fruit	Percent feeding	Mean number per fruit
Bowman FSA	55	2.55 ± 0.79a	35	1.25 ± 0.43ab
Krazy Glue	60	4.00 ± 1.20a	15	0.20 ± 0.12b
Loctite	65	3.10 ± 0.77a	35	0.65 ± 0.27ab
Water	65	4.40 ± 1.18a	50	1.95 ± 0.63a

Means followed by the same letter within a column are not statistically different (Tukey test, $P < 0.05$).

Table 3. Total distance moved, mean walking velocity, and duration of movement of multivoltine plum curculio adults over a 15-min period, 24 and 48 h after application of three commercially available glues to the pronotum

Treatment	Total distance moved ± SE (cm)	Duration ± SE (s)	Mean velocity ± SE (cm/s)
24 h			
Bowman FSA	45.77 ± 15.06a	54.59 ± 20.72a	0.78 ± 0.11a
Krazy Glue	35.68 ± 15.08a	33.89 ± 12.55a	0.84 ± 0.11a
Loctite	23.70 ± 6.02a	42.77 ± 19.74a	0.80 ± 0.11a
Water	72.89 ± 22.33a	70.56 ± 16.96a	0.90 ± 0.07a
48 h			
Bowman FSA	64.67 ± 19.61a	62.50 ± 16.02a	0.81 ± 0.09a
Krazy Glue	57.61 ± 10.31a	70.32 ± 15.16a	0.72 ± 0.08a
Loctite	75.92 ± 29.83a	72.36 ± 21.55a	0.84 ± 0.11a
Water	80.07 ± 21.39a	75.70 ± 16.52a	1.03 ± 0.09a

Means followed by the same letter within a column are not statistically different (Tukey test, $P < 0.05$).

Table 4. Percentage ($N = 40$) of adult multivoltine plum curculios that climbed <20 cm or reached the top (20 cm) of a vertical cylinder and mean distance climbed (cm) over a maximum period of 5 min after application of three commercially available cyanoacrylate glues to the pronotum

Treatment	Percent climbed <20 cm	Percent climbed 20 cm	Mean distance climbed ± SE (cm)
Bowman FSA	45.0	15.0	18.23 ± 8.28a
Krazy Glue	40.0	15.0	6.60 ± 1.85a
Loctite	37.5	12.5	6.50 ± 2.46a
Water	32.5	2.5	2.90 ± 0.99a

Means followed by the same letter within a column are not statistically different (Tukey test, $P < 0.05$).

Discussion

Survivorship

The three commercial formulations of cyanoacrylate glues tested were not toxic to the Colorado potato beetle or the plum curculio but were highly toxic to the corn rootworm. To the best of our knowledge, this is the first report of such a rapid and high level of mortality in an insect after the application of cyanoacrylate glues. Cyanoacrylate glues have been applied directly on Carabidae (Mascanzoni and Wallin 1986, Wallin and Ekbom 1994), Coccinellidae (Boiteau and Colpitts 2004), Curculionidae (Brazee et al. 2005), and Odonata (Wikelski et al. 2006), with no mention of important mortality. More recently, one of us (F.M.) used Bowman FSA glue to mount tags on the alfalfa snout beetle (Coleoptera: Curculionidae) with no lethal effect or obvious behavioral change.

The absence of toxicity of the glue with the Colorado potato beetle and the plum curculio may, in the same manner as insecticides, be attributable to a lower cuticle permeability than with the corn rootworm. Low cuticular permeability, associated to cuticle structure, chemical composition, or even thickness, is one of the modes of insect resistance to insecticides (Medina et al. 2002, Ahmad et al. 2006).

In a preliminary test carried with corn rootworm collected in late July–August 2003, the survival rate was 15–80% after 24 h with cyanoacrylate glues compared with 90% with the control beetles (unpublished data). This raises the possibility that the susceptibility of the corn rootworm to the cyanoacrylate glues varies depending on the time of the season.

Feeding Activity and Mobility

The absence of change in the average level of defoliation of potato plants and the visual assessment of the mobility of the adult suggest that the adhesives had no sublethal effect on the Colorado potato beetle. Similarly, the distance traveled horizontally, the time spent moving, and the speed of travel for the multivoltine plum curculios were essentially not affected by the glues. The distance traveled vertically, the time spent moving, and the speed of travel for the multivoltine plum curculios was not affected by the glues either. The average number of fruit punctures by female multivoltine plum curculio was not affected by the glues tested, but feeding by the male was reduced. Not enough corn rootworms survived to justify an experimental assessment of changes in behavior after treatment with glues. However, corn rootworms were visibly affected by the glue and became immobile or their movement became erratic until death.

Radar Tag Attachment

All formulations of cyanoacrylate tested provided a strong and durable bond of the radar tag. It was found through trial and error (Boiteau unpublished data), that sanding the pronotum tergum of the Colorado potato beetle provided a longer lasting bond. This is not possible with the plum curculio whose pronotum is smaller with an irregular surface or the corn rootworm whose pronotum is softer. Nevertheless the bond was also excellent for the plum curculio even without the sanding. The strength of the bond was lower with the Northern plum curculio than with the Colorado potato beetle, with 49.5% of the tags remaining on over the first 4 d and 36.5% on day 5. Regardless of the glue, the 50% of the tags that fell off did so over the first 24 h and the rest stayed on for at least 4 d. We speculate that the positioning of the proximal end of the wire and the droplet of glue among the asperities of the pronotum has a strong impact on the quality of the bond. A 24-h waiting period before releasing the plum curculio would ensure that relatively long-term/long distance tracking can be reliably carried out.

Alternative attachment methods will be required for the two corn rootworm species because of their sensitivity to the cyanoacrylate glues. Williams et al. (2004), having found no glue sticking adequately to the waxy cuticle of *Anoplophora glabripennis* (Cerambycidae), mounted a harmonic radar tag dorsally

Table 5. Survival (% ± SE) of Western ($N = 50$) and Northern ($N = 50$) corn rootworms 4, 16, 40, and 64 h after application of three commercially available glues and water on the pronotum

Treatment	Western corn rootworm				Northern corn rootworm			
	4 h	16 h	40 h	64 h	4 h	16 h	40 h	64 h
Krazy Glue	18 ± 5.83	6 ± 4.00	2 ± 2.00	2 ± 2.00a	6 ± 2.45	0	0	0b
Loctite	30 ± 7.07	12 ± 4.90	6 ± 2.45	6 ± 2.45a	20 ± 8.37	4 ± 2.45	2 ± 2.00	0b
Bowman FSA	4 ± 2.45	2 ± 2.00	0	0a	0	0	0	0b
Water	100 ± 0.00	90 ± 4.47	90 ± 4.47	85 ± 5.10a	100 ± 0.00	92 ± 2.00	86 ± 2.45	84 ± 2.45a

Means followed by the same letter within a column are not statistically different (Tukey test, $P < 0.05$).

on the prothorax and transversely to the beetle's long axis by first tying it to a length of dental floss and then tying the dental floss as a collar around the prothorax with the diode centered and the wires extending laterally. A similar procedure was developed by one of us (F.M.) for the corn rootworm. A fishing monofilament (Orvis 8X, 0.08 mm diameter) was used because of its lightweight and resistance to twisting. The monofilament is passed through the transponder loop, and a noose (slip knot or lasso) is made with the ends. The lasso is slipped over the corn rootworm's head and tightened up between the first and second thoracic segments. The slip knot is fixed in place by melting the knot ends with a heated 00 insect pin.

The ideal bonding agent for radar tags would share most of the properties identified by Hagler and Jackson (2001) for an ideal insect marking material: durable, inexpensive, nontoxic, easily applied, and without effect on normal behavior. More research is needed to find the ideal adhesive for use with harmonic radar tags, but the results showed that cyanoacrylate glues possess many of the properties sought after when used with the Colorado potato beetle or the plum curculio. The surprisingly high toxicity of the same materials against the corn rootworms is a reminder of the importance of carrying out preliminary studies on all the materials involved before choosing a method of tagging insects, because any given procedure or material is unlikely to be applicable for all species or research purposes.

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